Subcellular mRNA localization regulates ribosome biogenesis in migrating cells

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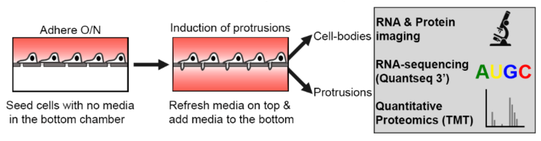
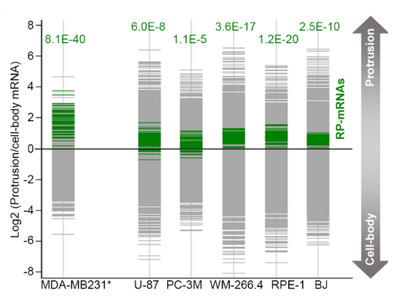
1: Introduction

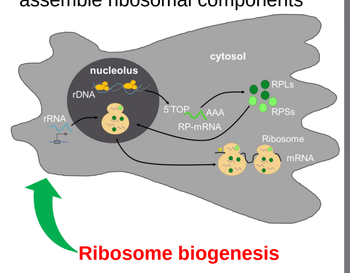
A. We observed that ribosomal proteins (RP)-mRNAs were enriched in protrusions of multiple cell lines



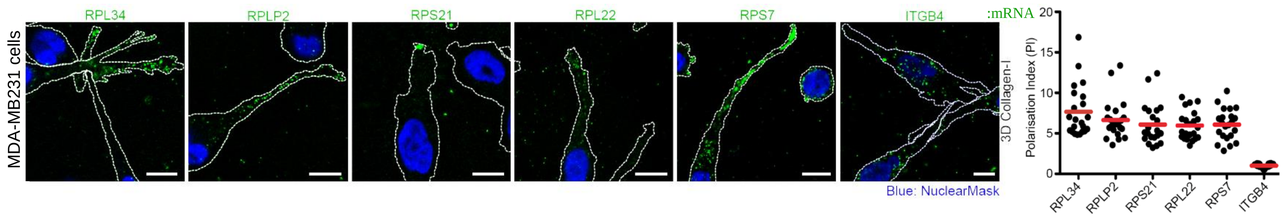
Cell-line:	MDA-MB231	U-87	PC-3M	WM-266.4	RPE-1	BJ
Tissue of origin:	Breast	Brain	Prostate	Skin	Retina	Foreskin
Cell-type:	Adenocarcinoma	Glioma	Adenocarcinoma	Melanoma	Pigment epithelial	Fibroblast
Malignancy:	Cancerous				Normal	



B. This is a paradoxical observation because RPs migrate to nucleus to assemble ribosomal components

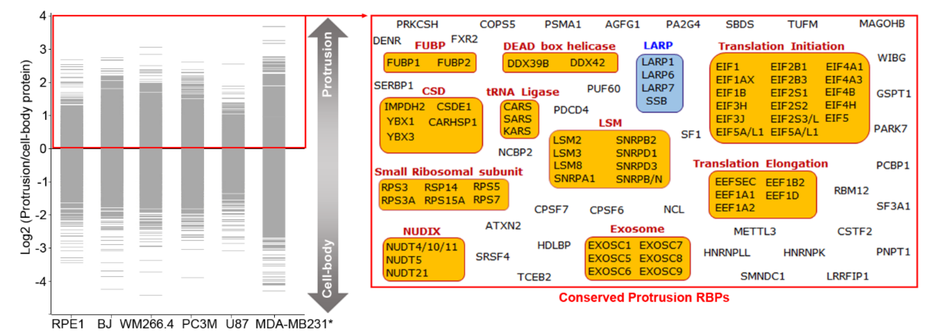


C. We validated our RNA-seq by microscopy and observed that RP-mRNAs are enrich in the periphery of actively migrating cells through 3D collagen-I

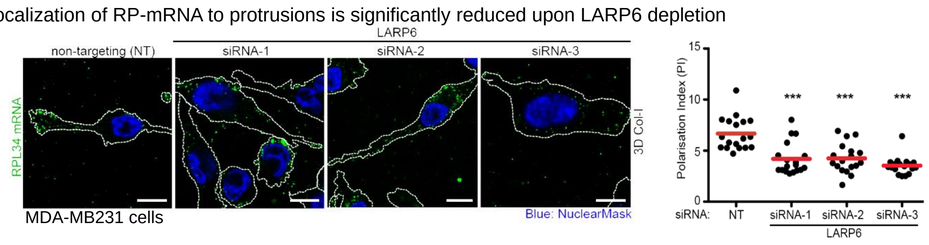


2: Protrusion-enriched LARP6 RBP mediates RP-mRNA localization

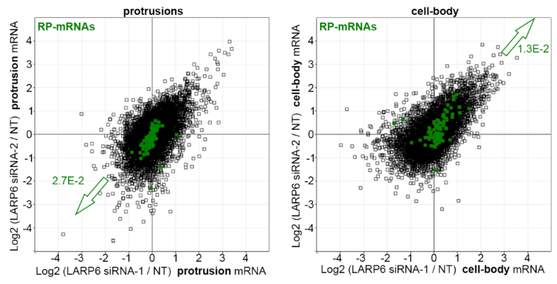
A. Protrusion-enriched RBPs belonging to same functional families are grouped. LARP family members are inside blue box



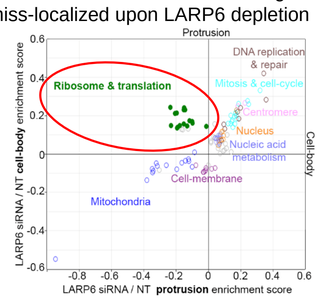
B. Localization of RP-mRNA to protrusions is significantly reduced upon LARP6 depletion



C. Depletion of LARP6 by two siRNAs decreases RP-mRNAs in protrusions

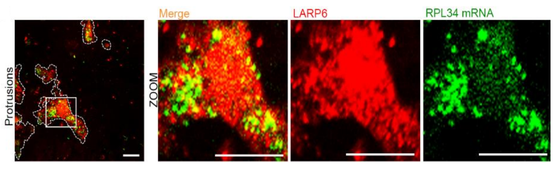


D. RP-mRNA and translation categories are miss-localized upon LARP6 depletion

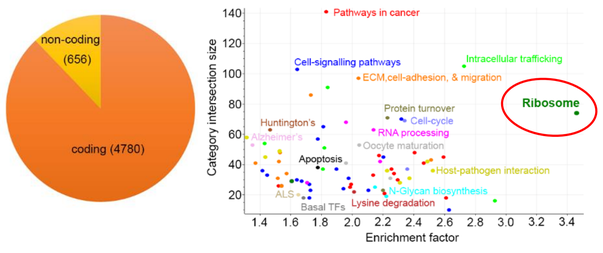


3 : Transcriptome-wide iCLIP reveals direct binding of LARP6 to RP-mRNAs

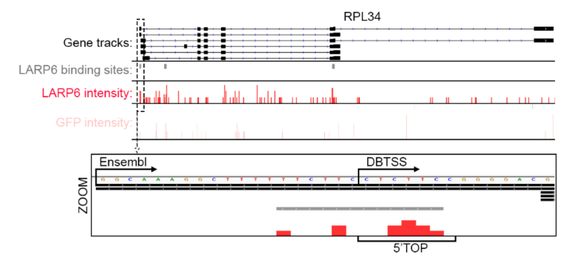
A. RP-mRNAs co-localize with LARP6 in protrusions



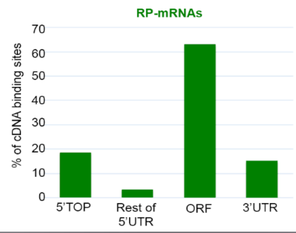
B. iCLIP reveals transcript-wide LARP6 binding targets, being RP-mRNAs the most enriched category



C. Representative RP-mRNA containing multiple LARP6 iCLIP peaks

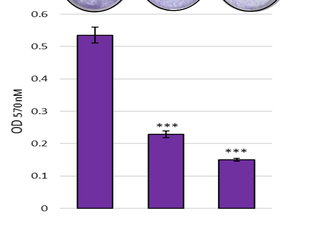


D. Distribution of LARP6 binding regions within RP-mRNAs

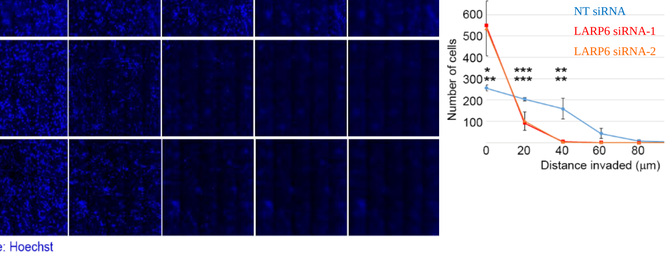


4 : LARP6 binding to STRAP is critical for its protrusion localization

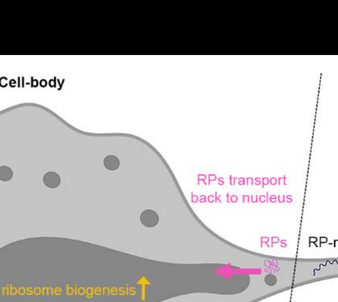
A. 35 proteins interact with GFP-LARP6



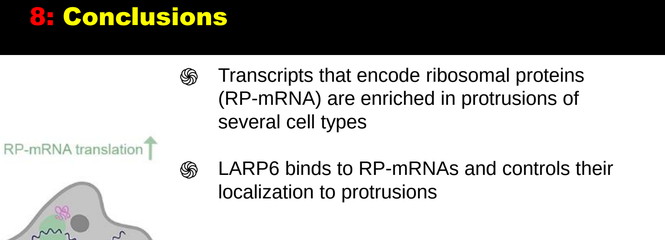
C. STRAP deletion reduces LARP6 enrichment in protrusions



B. STRAP binding to LARP6 is RNA-independent

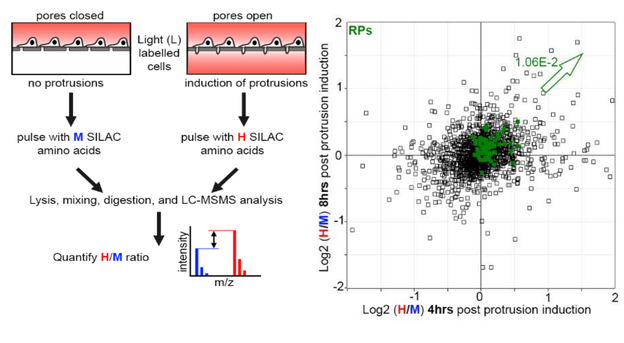


D. STRAP deletion decreases RP-mRNA enrichment in protrusions

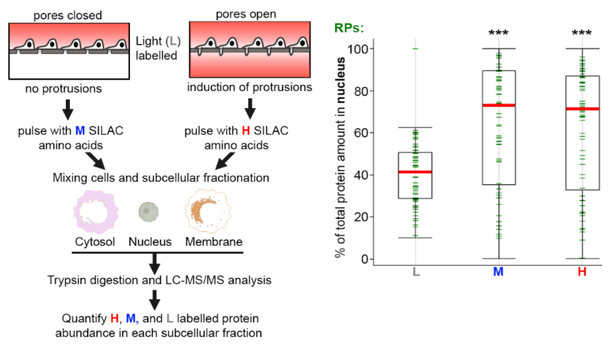


5 : LARP6-dependent RP-mRNA localization to protrusion enhances RP synthesis and canonical ribosome biogenesis

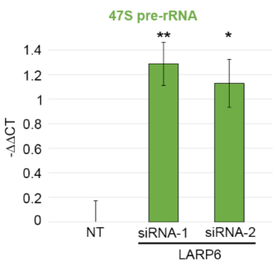
A. RPs translation is enhanced upon protrusion formation



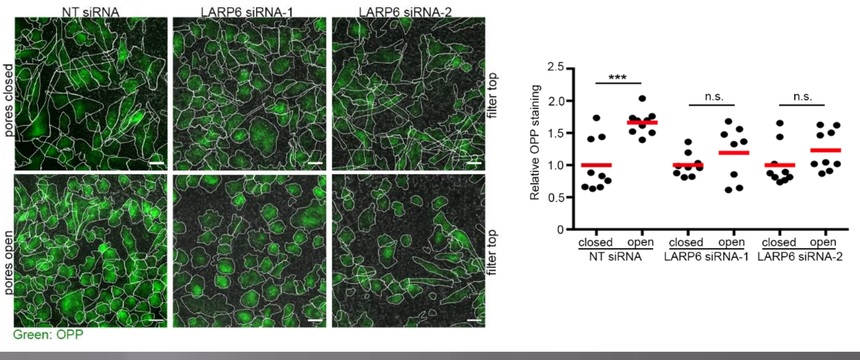
B. Newly synthesized RPs accumulate in the nucleus



C. LARP6 depletion perturbs pre-rRNA processing



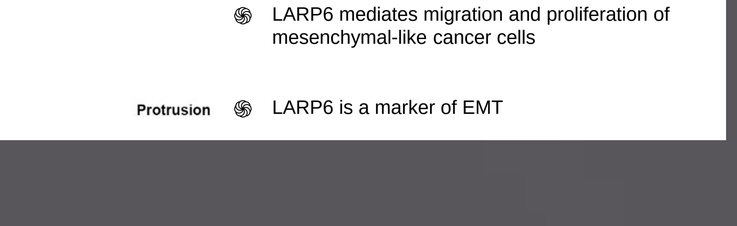
D. Enhancement of protein synthesis upon protrusion formation is inhibited by LARP6 depletion



6: LARP6 is important for proliferation and migration of mesenchymal-like cancer cells

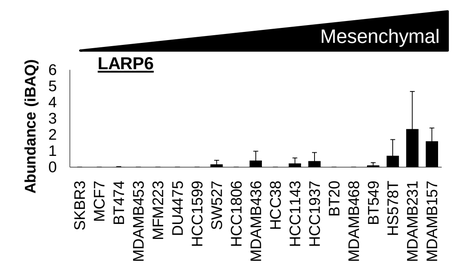
A. LARP6 depletion reduces proliferation

B. LARP6 depletion inhibits 3D migration



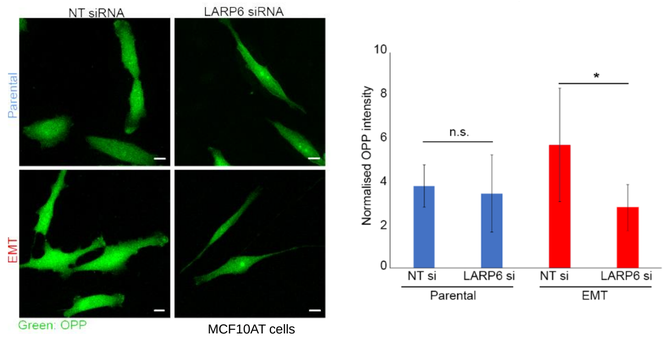
7: Enhanced expression of LARP6 in cancer is associated with EMT

A. LARP6 in triple-negative breast cancer cells lines

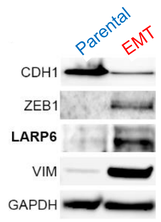


Proteomes of twenty human breast cell lines from Lawrence *et at.*

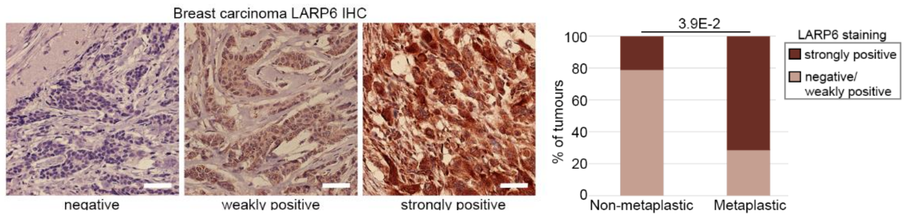
B. EMT induction enhances protein synthesis in a LARP6-dependent manner



C. EMT regulates LARP6 expression



D. LARP6 strongly-positive tumours are enriched in metaplastic carcinomas



8: Conclusions

- Transcripts that encode ribosomal proteins (RP-mRNA) are enriched in protrusions of several cell types
- LARP6 binds to RP-mRNAs and controls their localization to protrusions
- STRAP binding to LARP6 is independent of RNA and controls LARP6 localization to protrusions
- RP-mRNA localization to protrusions enhances RP synthesis and overall protein synthesis
- LARP6 mediates migration and proliferation of mesenchymal-like cancer cells
- LARP6 is a marker of EMT